NMR spectrum, δ, ppm: quartet at 3.75 (CH_2OH), singlets and doublets in the 0.6-1.7 region ($CH_3-\overset{|}{\underset{|}{C}}-$; $CH_3-\underset{|}{CH}-$).

SUMMARY

Extraction of the herb Ledum palustre with liquefied carbon dioxide and Khladon-11 has yielded 10 individual substances belonging to various classes and various groups of organic compounds: hydrocarbons, alcohols, ketones, monoterpenes, sesquiterpenes, and flavonoids.

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SOME BACTERICIDAL PROPERTIES OF OLIGOPEPTIDES AND REGULAR POLYPEPTIDES INCLUDING LYSINE AND ORNITHINE RESIDUES

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Histones take part as repressors in the regulation of gene activity, which is determined by their chemical nature: a high content of lysine and arginine. It is possible that the appreciable bactericidal activity of histones in relation to various microbes of the coli group and micrococci is also connected with this circumstance [1]. The antimicrobial mechanism of the action of histones has not yet been studied, but in view of their polycation-exchange properties it may be assumed that they are capable in some way of deforming membrane structures in microorganisms and of being nonspecific repressors.

Still more pronounced bactericidal properties in relation to certain bacteria are possessed by poly(α-amino acid)s containing lysine and arginine residues, and also their copolymers. Thus, copolymers containing ornithine, lysine, and arginine residues are active compounds not only for E. coli but also for Staphylococcus aureus [1]. The most active known antibacterial copolymer is a copolymer of leucine and ornithine in a ratio of 1:1. Its activity is 5 μg/ml [2]. The polypeptide H-(Leu-Orn-Leu)$_n$-OH possesses a similar activity [2].

The impression has been created that bactericidal properties depend on the primary structure of the polypeptide. To investigate this question we have obtained a series of oligopeptides containing lysine residues and have used regular polypeptides obtained previously, the synthesis of which has been described elsewhere [3-5]. The minimum concentrations of the oligo- and polypeptides possessing antibacterial properties are given below, all the other polypeptides possessing antibacterial activity at concentrations greater than 10 mg/ml:

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Oligopeptides and regular polypeptides	Mean molecular weight of the polypeptide	Bactericidal Activity of the compound, mg/ml
H-Lys_6-OCH_3	—	5,0
H-Lys_8-OCH_3	—	2,5
H-Ala-Lys_4-Ala-OCH_3	—	10,0
H-Ala-Lys_6-Ala-OCH_3	—	—
H-$(Lys\text{-}Ala_2)_n$-OH	—	—
H-$(Lys\text{-}Ala_2)_n$-OH	—	—
H-$(Lys_2\text{-}Ala)_n$-OH	—	—
H-$(Lys_2\text{-}Ala_2)_n$-OH	—	—
H-$(Lys_3\text{-}Ala)_n$-OH	—	—
H-$(Ala_3\text{-}Lys)_n$-OH	—	—
H-$(Lys\text{-}Pro)_n$-OH	5,800	12,85
H-$(Lys\text{-}Pro_2)_n$-OH	4,500	1,43
H-$(Lys_2\text{-}Pro)_n$-OH	1,400	1,25
H-$(Lys_3\text{-}Pro)_n$-OH	5,000	0,67
H-$(Gly\text{-}Pro\text{-}Lys)_n$-OH	—	—
H-$(Lys\text{-}Gly_2)_n$-OH	—	—
H-$(Lys_2\text{-}Gly_2)_n$-OH	—	—
H-$(Lys_3\text{-}Gly)_n$-OH	3,000	0,68
H-$(Orn_3\text{-}Gly)_n$-OH	3,000	0,62
H-$(Orn_2\text{-}Ala_2)_n$-OH	5,000	5,0
H-$(Orn_2\text{-}Gly_2)_n$-OH		

It follows from the figures given that the activity of the oligo- and polypeptides that we have obtained in relation to St. aureus is lower than the activity given by Fridken et al. [2].

Since we had available a set of regular polypeptides with various amounts of lysine, ornithine, proline, alanine, and glycine residues, this enabled us to detect a characteristic dependence of bactericidal properties on their amounts and positions. We have established that an increase in the amount of lysine residues in the oligo- and polypeptides leads to a rise in antimicrobial activity. It is obvious that an important role is played by aliphatic amino-acid residues. Thus, while the polypeptide H-$(Lys_3\text{-}Ala)_n$-OH does not suppress the growth of St. aureus in a concentration of 10 mg/ml, when the alanine residue is replaced by a proline or glycine residue the activity rises to 0.68 mg/ml (compounds 14, 18).

Thus, it may be concluded that proline residues play an extremely important role in the manifestation of bactericidal properties. So far as concerns glycine and alanine residues, no characteristic features whatever have been observed in their influence.

There is no doubt that such compounds may prove to be very interesting chemotherapeutic agents, and it is not excluded that the choice of appropriate amino-acid sequences will considerably enhance the biological activity and broaden the spectrum of their action.

EXPERIMENTAL

The methyl esters of the oligopeptides were obtained in the following way. Dry hydrogen bromide was passed for 40 minutes through a solution of 1 g of the methyl ester of an α-tert-butoxycarbonyl-N^{ε}-benzyloxycarbonylpeptide in 20 ml of glacial acetic acid. Then the reaction product was precipitated with ether, filtered off, and reprecipitated from ethanol with ether 3-4 times, after which the solid residue was triturated with hexane, filtered off, and dried over caustic soda.

In order to free it from hydrogen bromide, the hydrobromide of the methyl ester of the peptide was dissolved in ethanol and the solution was passed through a column (14 × 100 mm) containing IRA-401 ion-exchange resin in the OH^- form. The solution was evaporated and the residue was triturated with ethane and with hexane.

The purities of the compounds obtained were checked by paper electrophoresis. The completeness of the removal of the side-chain-protecting N^{ε}-benzyloxycarbonyl groups was determined spectrographically at 258 nm.

Determination of the Bactericidal Properties of the Oligo- and Polypeptides. A weighed sample (20 mg) of an oligopeptide or a polypeptide was dissolved in 5 ml of distilled water. Then twofold dilutions of the substances with broth giving factors of 2, 4, 8, 16, etc., were prepared. As the strain to be inhibited we used fresh Staphylococcus aureus with all its pronounced characteristics of pathogenicity. The experiments were carried out with an agar culture incubated in a thermostat at 37°C for 18 h. The microbial suspension was diluted with

physiological solution to a concentration of 200,000 microbial cells per ml, and 0.2 ml of it was added to the working solution and also to a control culture (meat-peptone broth and the above-mentioned concentration of the culture). The inoculated preparations were incubated in a thermostat at 37°C for 18 h and the growth of the incubated culture was examined.

SUMMARY

Oligopeptides and polypeptides of the sequences Lys_6-, Ala-Lys_4-Ala, Lys_8-, Orn_2-Ala_2-, Lys_3-Pro-, Lys_3-Gly-, Orn_3-Gly-, Lys-Pro-, Lys-Pro_2-, Lys_2-Pro-, Lys_3-Ala- possess bactericidal properties in relation to a strain of the bacterium Staphylococcus aureus.

It has been shown that with an increase in the amount of lysine and ornithine residues in the polypeptide chain the bactericidal properties increase; proline residues play a fundamental part in the manifestation of these properties.

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